PLASMA BIOMARKERS PREDICTION ON CHRONIC OBSTRUCTIVE PULMONARY DISEASE PROGRESSION

by

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ABSTRACT

Background:

Chronic Obstructive Pulmonary Disease (COPD) is a progressive disease resulting from persistent airflow limitation. The severity of COPD is categorized based on forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the ratio of FEV_1/FVC by pulmonary function test. The rate of change in FEV_1 could influence COPD management and treatment, however, it varies significantly across people. Early disease treatment applied on COPD patients with rapid decline in FEV_1 could prevent patients from early exacerbation in lung function failure. We hypothesize that plasma biomarkers are associated with COPD progression and could identify patients with rapid decline in FEV_1.

Methods:

A cohort of 313 former smokers was followed for 2 years. Pulmonary function test (for FEV_1) and computed tomography (for Frac-950 and multiple lobes based on visual emphysema score, MLVS) were applied to all subjects when entering the study and at the 2-year follow-up visit. Peripheral plasma samples were collected when entering the study and plasma levels of 17 biomarkers were recorded with the samples.

Results:

Plasma levels of C-reactive protein (CRP) and matrix metalloproteinase-1 (MMP1) are significant positively associated with continuous FEV_1 percent predicted change. Plasma level of soluble intercellular adhesion molecule-1 (sICAM1) is significant in estimating continuous Frac-950 change positively and plasma levels of sICAM1 and MMP1 are significant in estimating Frac-950 rapid decline positively. Plasma level of tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is significant negatively and plasma level of tissue inhibitor of metalloproteinases-2 (TIMP2) is significant positively associated with continuous MLVS change, and plasma levels of sclerostin (SOST), tissue inhibitor of metalloproteinases-1 (TIMP1) and TIMP2 are significant positively associated with MLVS rapid decline. P-values less than 0.05 are considered significant.

Conclusions:

Peripheral plasma biomarkers are associated with COPD progression but variations exist. The levels of biomarkers along with baseline lung function could estimate the COPD patients with future rapid decline in lung function.

Public Health Significance:

Early treatment on those rapid decliners could reduce disease exacerbation and COPD comorbidities.

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1.0 INTRODUCTION TO COPD

1.1 DEFINITION OF COPD

Chronic obstructive pulmonary disease (COPD) is a progressive disease causing persistent airflow limitation. It forms primarily due to exposure to noxious particles or gases and results in an enhanced chronic inflammatory response in the airways and the lung [1].

COPD is caused by a mixture of small airway disease (known as obstructive bronchiolitis) and lung parenchymal destruction (known as emphysema) [1]. Obstructive bronchiolitis is a result of chronic inflammation which changes the structure by narrowing small airways in lung, including lumen collapse, alveolar attachments loss, mucus increase and wall fibrosis [2]. Emphysema is defined as permanent destructive enlargement of the airspace distal to the terminal bronchiole, affecting both bronchioles and alveoli. Some COPD patients may be diagnosed with both obstructive bronchiolitis and emphysema while others only develop one of the two disease manifestations [3]. The walls of bronchiole are thickened and the alveoli are enlarged in COPD lung compared with normal lung.

1.2 ETIOLOGY OF COPD

1.2.1 Risk factors

Among the noxious particles and gases, cigarette smoking is the most commonly recognized for causing COPD [4, 5]. Approximately 75% of COPD cases are attributed to cigarette smoking [6]. The risk of developing COPD and the severity of COPD in smokers increase with the amount of cigarette smoked [7]. Persistent smokers (odds ratio (OR) 4.56, 95% confidence interval (CI) 2.67~7.79) and restart smokers who quit smoking and restarted (OR 3.57, 95% CI 1.06~12.00), are more likely to develop COPD than persistent ex-smokers those quit and did not restart (OR 1.03, 95% CI 0.58~1.83), compare to non-smokers [8]. Passive exposure to cigarette smoke may also contribute to COPD. Exposure to environmental cigarette smoke was related to a greater risk of COPD (OR 1.36, 95% CI 1.002-1.84) compared to those without the exposure [9].

In addition to cigarette smoking, other noxious particles from occupational related exposures, in-door and out-door air pollution demonstrate significant association with COPD. Occupational exposures, such as coal mining, hard-rock mining, concrete-manufacturing etc., may account for 15% of COPD cases [10]. Occupational exposure to dusts from agricultural industry and mining is associated with increased odds of COPD (OR 1.53, 95% CI 1.17~2.08) compared to those not exposed [11]. Indoor and outdoor pollution associated with COPD include pollutants from industry, heating, traffic, cooking and other sources. Studies provide evidence of association of indoor and outdoor pollution with COPD diagnosis or severity. A positive association between exposure to solid fuel and COPD diagnosis has been observed (OR 2.80, 95% CI 1.85~4.0) [12]. Solid fuel is the solid material for producing energy and providing heat

when burning, including wood, coal, peat and pellets made from grains. Higher PM_{10} (particulate matter of less than 10 mm) within 8 km distance from home address was related to an increased risk for COPD (OR, 1.33 per 7 mg/m3; 95% CI, 1.03–1.72) [13]. Burning of biomass fuel, mainly woody fuel and animal waste, increases development of COPD (OR 1.95, 95% CI 1.55~2.4) [14].

Genetic factors are also considered to play an important role in COPD development. The most documented genetic risk factor is alpha-1 antitrypsin deficiency, which is a major circulating inhibitor of serine proteases [15]. Apha-1 antitrypsin deficiency is most commonly seen in individuals of Northern European origin [16]. Although apha-1 antitrypsin deficiency is relatively rare, it illustrates the effect of genes on COPD development.

Besides the inhaled particles and genetic factors, there is evidence of sex differences in COPD diagnosis. COPD was considered to be a male dominated disease because men had a higher cigarette smoking rate and more exposure to occupational hazards than women. However, the percentage of men versus women diagnosed with COPD is equalizing. Although the age adjusted mortality of COPD in men was 1.3 times greater than in women, the absolute death numbers of women increased more than that of men, from 58,729 in 1999 to 70,066 in 2009 compared to an increase from 60,795 in 1999 to 63,899 in 2009 respectively [17]. Potential reasons for the increasing cases in women may be a combined effect of increased tobacco consumption among women in developed countries, higher risk of exposure to indoor air pollution in developing countries, and increased amount of women working in historically male dominant jobs which are exposed to occupational dusts [18].

Pulmonary function declines during normal aging process. Moreover, cumulative exposures to inhaled particles during aging process increase the risk of developing COPD. A

systematic review and meta-analysis, including studies from 28 countries between 1990 and 2004, demonstrate the higher prevalence of COPD in those above 40 years old in comparison with those below 40 [19.

Hu et al studied the effect of nutrition and diet on developing COPD [20]. They found that a diet high in antioxidants may be a protective factor in the development of COPD and higher plasma vitamin C levels were associated with higher pulmonary function.

1.2.2 Pathology

COPD results from persistent inflammation caused by inhaled irritants. The inflammation increases the number of inflammatory cells, including neutrophils, lymphocytes and macrophages. These inflammatory cells interact with structural cells in the airways and parenchyma by breaking down the balance between proteinases and anti-proteinases and the balance between oxidative and anti-oxidative and results in COPD [1, 2]. Oxidants and proteinases, generated by irritants and derived from inflammatory cells, increase oxidative stress and proteinases activity and accelerate inflammation.

1.3 BURDEN OF COPD

1.3.1 Prevalence, Mortality and Morbidity

COPD affects more than 400 million people worldwide, with approximately 65 million people have moderate to severe COPD [1, 21]. The burden of disease epidemiology estimates

that the average prevalence of moderate or severe COPD is around 10.1% and sex specific rates are 11.8% for men and 8.5% for women [22]. In United States, 12.7 million adults over 18 years old had COPD in 2011 and an additional 24 million were suspected to have undiagnosed COPD [23].

According to World Health Organization global burden of disease study 2000, COPD accounts for 2.75 million deaths worldwide [24]. Chronic lower respiratory disease, primarily COPD, was the third leading cause of death in the United States and responsible for 149,205 deaths in 2011 [25].

The World Health Organization global burden of disease study states that COPD is the fourth leading cause of mortality and will be the third leading cause of mortality worldwide by 2030 [26].

1.3.2 Economic and Social Burden of COPD

Both direct and indirect costs are high for COPD. In the United States, almost $49.9 billion was spent on COPD in 2010, including $29.5 billion in direct health care expenditures (value of health care for disease diagnosis and medical treatment), $8.0 billion in indirect morbidity costs (monetary consequences of disability and missed work) and $12.4 billion in indirect mortality costs (productivity lost due to early death) [27]. Worldwide, COPD is the twelfth leading cause of DALYs (Disability-Adjusted Life Year) lost in the world, responsible for 2.1% of the total in 1990. COPD is projected to be the seventh leading cause of DALYs lost by 2030 [28].

1.4 COMORBIDITIES OF COPD

COPD is a complex syndrome often developed in middle age along with diseases related to aging [29]. There is growing recognition that inflammation of COPD not only affect lung but also is harmful to non-pulmonary organs.

Patients with COPD have a higher prevalence of osteoporosis compared to health adults (32.5% versus 11.4% respectively, p-value<0.001) [30-34]. Bone mineral density value decreases as the severity of COPD and decrease in pulmonary function associates with osteoporosis (OR=0.46, p-value=0.04) [35]. Bone attenuation positively correlates with pulmonary function (r=0.06, p-value=0.01) and negatively correlates with extent of emphysema (r=-0.09, p-value<0.001) [36]. Osteoporosis is disease with increased bone fragility due to decreased bone density. Besides common risk factors, aging and smoking, COPD influences on vitamin D level. A group of 44 males with COPD show 27% of them have lower serum concentration of 25 hydroxyvitamin D (25(OH) vitamin D) compared to the lower limit of that for healthy subjects [37]. The treatment for COPD can also influence osteoporosis. Oral corticosteroid, a medicine for COPD, is associated with higher rate of non-vertebral fracture (relative rate (RR) =1.33, 95% CI 1.29-1.38), hip fracture (RR=1.61, 95% CI 1.47-1.76) and vertebral fracture (RR=2.60, 95% CI 2.31-2.92) [38].

The prevalence of skeletal muscle weakness is shown to be higher in COPD patients (32.3% versus 8.0%, p-value<0.05) and higher prevalence rate is reported in severe COPD cases (OR 2.2, 95% CI 1.3-3.7) [39]. The prevalence of obesity or being overweight was increased in COPD patients than in those without airflow limitation in a population based sample (29.4% versus 24.3%; and 44.7% versus 43.0%, respectively; p-values<0.05) [40]. COPD patients tend to have a higher risk for cognitive impairment (36% versus 12%, p-value=0.007) and dementia

(42% versus 14%, p-value<0.05) [41, 42]. A nationwide population-based study estimates that the overall incidence rate of Parkinson's disease is 57% higher in the COPD patients than in non-COPD controls [43]. The pulmonary and cardiovascular systems are intimately related [44]. Evidence shows COPD patients have increased arterial stiffness which could be measured by pulse wave velocity (pulse wave velocity 11.4 m/s in COPD patients versus 8.95 m/s in healthy subjects, p-value<0.0001) [45]. Patients with COPD have higher risk of developing coronary artery disease (OR 2.0, 95% CI 1.5-2.5), angina (OR 2.1, 95% CI 1.6-2.7), myocardial infarction (OR 2.2, 95% CI 1.7-2.8), stroke (OR 1.5, 95% CI 1.1-2.1), and congestive heart failure (OR 3.9, 95% CI 2.8-5.5) [46]. Those associations are seen worldwide [47-61].

Comorbidities with COPD are common. A review on COPD comorbidities shows 97.7% patients have one or more comorbidities and 53.5% patients with four or more comorbidities [62]. Up to 25% of the population 65 years and older with COPD has two comorbid conditions and up to 17% have three [63]. On average, COPD patients aged 65 years and older have three or more chronic conditions [64]. The increasing number of comorbidities decreases quality of life (r=0.45 with poor quality of life, p-value<0.05) and self-reported health status [65, 66]. The comorbidities of COPD may contribute to increased morbidity and mortality [67, 68], and are positively associated with increased exacerbations and hospitalizations [69-74]. Anant RC Patel and John R Hurst's research reported that more patients with COPD may die from comorbidities of COPD than they do from COPD itself [75].

1.5 CLINICAL PHENOTYPING OF COPD

1.5.1 Spirometry

Clinical diagnosis of COPD is determined based on spirometry testing. Spirometry is the most common pulmonary function test measuring lung function, specifically the amount and/or speed of air that can be inhaled and exhaled. When it is used to confirm COPD diagnosis, it is generally carried out with pre- and post-bronchodilator usage, a medication to open up the airways [76]. Two main components are recorded: the greatest volume of air that can be exhaled in a single deep breath (forced vital capacity, FVC) and the greatest volume of air that can be exhaled in the first second of a breath (forced expiratory volume in one second, FEV_1) [77]. The ratio of these two measurements is calculated (FEV_1/FVC).

The values of FEV_1 and FVC depend on age, sex, height and ethnicity. In healthy adults, the ratio of FEV_1 to FVC is approximately 0.75~0.80, while in patients with obstructive disease, FEV_1 is diminished because of increased airway resistance to expiratory flow resulted from the premature closure of airway in expiration [78]. Although both FEV_1 and FVC are reduced in COPD, FEV_1 is affected more because of the increased airway resistance. A post-bronchodilator FEV_1/FVC <0.70 indicates that the patient has a persistent airflow limitation and is the diagnostic criteria for COPD [1].

The global initiative for obstructive lung disease (GOLD) divide COPD into four stages by disease severity using spirometry, mild, moderate, severe and very severe (Table 1 [1]).

Table 1 Spirometric Classification of COPD Severity Based on Post-Bronchodilator FEV_1

Mild	FEV_1/FVC <0.70 FEV_1 pp*>=80% predicted
Moderate	FEV_1/FVC<0.70 50%<= FEV_1 pp <80% predicted
Severe	FEV_1/FVC<0.70 30%<= FEV_1 pp <50% predicted
Very Severe	FEV_1/FVC<0.70 FEV_1 pp <30% predicted

* FEV_1 pp (FEV_1 percent predicted) is the FEV_1 of the patient divided by the average FEV_1 in the population for any person of similar age, sex, height and ethnicity [79].

1.5.2 Quantitative computed tomography (CT) measurement and semi-quantitative CT measurement

Traditional pulmonary function test, spirometry, is a measurement of airway dominant COPD and has a low sensitivity in diagnosing early stage of emphysema [80]. Around 30% of patients may develop emphysema before exhibiting detectable decline in a spirometry pulmonary function test [81]. There are two measurements for emphysema, one is quantitative CT and the other is semi-quantitative visual assessment based on CT image.

Computed tomography (CT) is an imaging procedure to describe inner body by X-ray. High-resolution CT scans have been proven to reflect microscopic emphysema [82]. Software calculates the emphysema index as the fraction of voxels less than −950 Houndsfield Unit (Frac-950) as a percent of total voxels identified as lung regions similar to the traditional density mask [83].

Multiple lobes based visual emphysema score system (MLVS) is a new system to score the degree of emphysema in the lung based on the CT image. It is an improvement of traditional used visual emphysema score and is based on a visual inspection of the CT image by

professional radiologist. To derive the MLVS, the left and right lung are divided into 6 segments with upper, middle and lower regions of the right lung and the upper, lingula and lower regions of the left lung. The upper and lower segments in each side of lung are assigned with weight 2 respectively, and middle and lingula segments with weight 1 respectively. The visual emphysema score of each segment is multiplied by the weight for that segment. Then sum of all segment-specific visual scores are then divided by 10 (the sum of weights) to derive the final MLVS value.

Although Frac-950 and MLVS were two ways of measuring emphysema, they do have differences. MLVS is calculated by professional radiologist while Frac-950 is derived automatically by computational methods. Sometimes, Frac-950 and MLVS score provide different information for the same patient. Radiologist may miss some of the emphysematous changes if the changes are too small and may rate a small MLVS score. Similary, structural abnormality may also lead an inaccurate rating of emphysema in the computation of Frac-950 score.

Frac-950 and MLVS are continuous variables. Higher values of Frac-950 and MLVS indicate more severe COPD.

1.6 DISEASE PROGRESSION IN COPD

A key feature of COPD is the accelerated decline in FEV_1. However, the rate of change in FEV_1 varies significantly. A study by Vestbo shows the mean rate of change in FEV_1 is a decline of 33 ml/year with a 59 ml/year standard deviation [84]. In addition, 38% of the study participants had an estimated rate of change of decline in FEV_1 by more than 40 ml per year,

31% declined by 21 to 40 ml per year, 23% declined 20 ml/year to increased 20 ml/year and 8% increased more than 20 ml/year (Figure1 [84]).

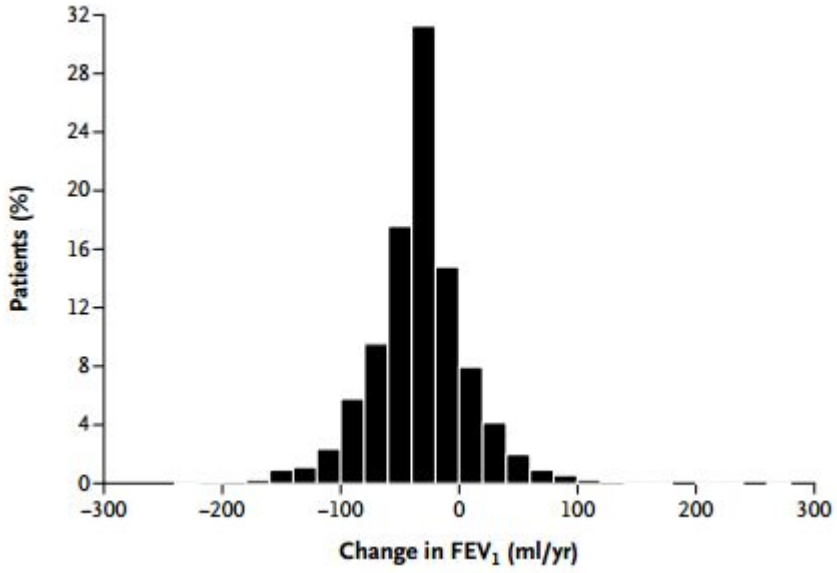

Figure 1. Distribution of Estimated Annual Rates of Change in FEV1 over a 3-Year Period in Patients with COPD

Knowing the predicted rate of change in FEV_1 could influence disease management and treatment. This could prevent early exacerbations in lung function failure, especially for the rapid decliners. Therefore, identifying patients with rapid decline in FEV_1 is important and urgent for COPD treatment.

1.7 PLASMA BIOMARKERS

Biomarkers may provide novel strategies for selecting patients with high risk of rapid decline in FEV1. Biomarkers may reflect disease progression [85]. Common sample sources of

biomarkers for COPD include exhaled breath, induced sputum, bronchoalveolar lavage fluid, lung biopsies, and peripheral blood. However, the first four measurements are invasive of the procedures, have poor reproducibility, and a lack of standardization of measurements. These methodological deficiencies limit their clinical application [86]. The focus of biomarker discovery has been regarding blood specimens as they are reliable, cost-effective and readily available in clinical settings [87].

Existing research indicates certain blood-based biomarkers are related to lung function and clinical outcomes such as exacerbations, morbidity and mortality [88]. Peripheral biomarkers associated with COPD clinical phenotype, disease severity and development have been reported including C-reactive protein (CRP), fibrinogen, surfactant protein D (SPD) and Clara cell protein (CCP) [89-94]. Increases in plasma level of CRP overtime are associated with decreases in FEV_1 % predicted [91]. Higher levels of fibrinogen are associated with COPD exacerbations and severe airflow limitation [92]. Elevated plasma levels of SPD were observed in COPD compared to controls [93].

As discussed in the pathology section, inflammation is a process including inflammatory cells and cytokines. Research shows that matrix metalloproteinase-12 (MMP12) degrades the extracellular matrix (ECM), for example elastin, and matrix metalloproteinase-9 (MMP9) degrades serine protease inhibitor, alpha-1 antiproteinase, during inflammation. Tissue Inhibitor of Metalloproteinases (TIMPs) inhibits the function of MMPs. Fluctuation in TIMPs affects MMPs. SPD and CCP are known lung specific protein involved in inflammation. CRP, Serum amyloid A (SAA) and human pentraxin-related protein 3 (hPTX3) belong to acute phase protein, a class of protein whose plasma concentration increase or decrease in response to inflammation. Soluble Intercellular Adhesion Molecule-1 (sICAM1) and Soluble Vascular cell Adhesion

Molecule-1 (sVCAM1) are two cell adhesion molecules that bind with ECM in cell adhesion process and activate or damage cell and endothelium. Tumor Necrosis Factor Receptor Type I (TNFRI), Tumor Necrosis Factor Receptor Type II (TNFRII) and TNF-Related Apoptosis-Inducing Ligand (TRAIL) are related to tumor necrosis factors (TNF) and apoptosis, which regulate the pathway implicated in resolution of inflammatory processes. Soluble Fas Receptor (sFas) is a protein and leads to cell death. Studies have demonstrated that TRAIL and sFas initiate inflammation besides the role as initiators of apoptosis [95]. Sclerostin (SOST), a glycoprotein, is associated with osteoporosis by anti-anabolic effects on bone formation. Osteoporosis and emphysema are epidemiologically associated disease of cigarette smoking. The finding that antigen-specific autoimmune response, anti-GRP 78 autoantibodies, are associated with osteoporosis and emphysema indicates SOST, proven to associated with osteoporosis, may associated with COPD inflammation [96]. Table 2 shows a list of the selected biomarkers we want to identify in COPD pathology process and COPD progression.

Table 2. Description of 17 Plasma Biomarkers

Biomarker Full Name	Abbreviation	Function
Matrix metalloproteinase-1	MMP1	proteinase related to inflammation
Matrix metalloproteinase-7	MMP7	proteinase related to inflammation
Tissue Inhibitor of Metalloproteinases-1	TIMP1	proteinase related to inflammation
Tissue Inhibitor of Metalloproteinases-2	TIMP2	proteinase related to inflammation
Tissue Inhibitor of Metalloproteinases-4	TIMP4	proteinase related to inflammation
Clara Cell Protein	CCP	lung-specific protein
Surfactant Protein D	SPD	lung-specific protein
C-Reactive Protein	CRP	inflammatory protein
Serum Amyloid A	SAA	inflammatory protein
Human Pentraxin-related Protein 3	hPTX3	inflammatory protein
Soluble Intercellular Adhesion Molecule-1	sICAM1	inflammatory protein
Soluble Vascular cell Adhesion Molecule-1	sVCAM1	inflammatory protein
Tumor Necrosis Factor Receptor Type I	TNFRI	induces apoptosis
Tumor Necrosis Factor Receptor Type II	TNFRII	induces apoptosis
TNF-Related Apoptosis-Inducing Ligand	TRAIL	induces apoptosis
Soluble Fas Receptor	sFas	inhibits apoptosis
Sclerostin	SOST	inhibits Wnt signal pathway

2.0 RATIONALE AND HYPOTHESIS

Former studies show the variation of COPD progression by FEV_1 and identify plasma biomarkers' association with COPD inflammation. However, few longitudinal studies of patient cohorts have provided the association between peripheral plasma biomarker and COPD progression. Therefore, we observe the three clinical phenotypes of COPD, FEV_1, Frac-950 and MLVS, on a group of former smokers. We believe that peripheral plasma level of biomarkers at beginning of the study could affect the change in clinical phenotypes of COPD and can estimate 2-year COPD progression.

3.0 STUDY DESIGN AND EXPERIMENTAL METHODS

3.1 STUDY POPULATION

Subjects were recruited from the existing Pittsburgh Specialized Center in Clinically Oriented Research (SCCOR) cohort. The SCCOR study focuses on advanced cellular and molecular investigations of lung tissue changes involved in COPD to increase understanding of disease progression. The subjects from the Pittsburgh SCCOR cohort consisted of former smokers with more than 10 pack-years smoked, who are older than 40 years old and without significant non-COPD related comorbidities at baseline.

Data collection for the baseline visit was conducted between July 2007 and November 2012. The 2-year follow-up visits were conducted at 2 years after the initial baseline visit, from July 2009 to November 2014. Institutional Review Board of the University of Pittsburgh approved this study.

3.2 CLINICAL PHENOTYPES AND DATA COLLECTION

3.2.1 Pulmonary Function Test

All participants performed standardized spirometry according to the European Respiratory Society guidelines, recording FEV_1 at baseline and at the 2-year follow-up visit. FEV_1 percent predicted (FEV_1 pp) value is calculated by dividing the individual's FEV_1 by the average FEV_1 in the population for any person of similar age, sex, height and ethnicity [79]. Smaller values of FEV_1 pp indicate more severe COPD. The continuous change of FEV_1 pp between first and second measurements for every participant was calculated by subtracting the baseline value from the 2-year follow-up value. A positive value of FEV_1 pp change indicates an improvement where COPD gets better. Based on clinical experience, we define an FEV_1 rapid decline if FEV_1 pp decreases by at least 5%.

3.2.2 Quantitative and semi-quantitative CT measurement

Each participant completed two chest CT scans, one at baseline and the other at the 2-year follow-up visit. Frac-950 was recorded for every participant for each visit. Greater values of Frac-950 indicates severe emphysema. The change in Frac-950 between baseline and 2-year follow-up for every participant was calculated by subtracting the baseline value from the 2-year follow-up value. An increase in continuous change in Frac-950 implies disease exacerbation. Based on clinical experience, we define a Frac-950 rapid decline if Frac-950 increases by at least 0.01.

MLVS was calculated for every participant by professional technician for each visit. Greater value of MLVS indicates severe emphysema. The change in MLVS between baseline and 2-year follow-up for every participant was calculated by subtracting the baseline value from the 2-year follow-up value. An increase in continuous change in MLVS implies disease exacerbation. Based on clinical experience, we define a MLVS rapid decline if MLVS increases by at least 0.5.

3.3 PLASMA BIOMARKERS

Plasma biomarkers samples were collected using citrated blood from subjects at baseline and banked at -80ºC. A total of 18 plasma biomarkers (shown in Table 2) were analyzed. TRAIL, sFas, SOST, TNFRI, TNFRII, CRP, SAA, sICAM1, sVCAM1 were analyzed using MSD multiplex assays (Meso Scale Discovery); MMP1, MMP7, TIMP1, TIMP2, TIMP4 were analyzed using Performance Assay. MMP and TIMP were analyzed using multi-plex Kits (R and D Systems) on a Luminex system, CCP and SPD were measured using ELISA (Biovendor R and D) and hTSP1 and hPTX3 were measured using ELISA duo set (R and D Systems). All of the assays were performed according to the manufacture's instruction. Analysis were performed in duplicates (ELISAs) or at least 15% of the samples on each assay plate were performed in duplicates (MSD and Luminex). Coefficient of variation was controlled at <15%.

3.4 STATISTICAL ANALYSIS

Continuous data were summarized as mean ± standard deviation and categorical data were summarized with percentages. Log-transform was applied to all biomarkers. P-values less than 0.05 are considered statistically significant.

3.4.1 Continuous Clinical Measurements

Univariate linear regression analysis was used to determine the associations of individual biomarker and the continuous changes of FEV_1 pp, Frac-950 and MLVS respectively. Multivariable regression analysis was used to determine the associations of individual plasma biomarkers with the continuous changes of FEV_1 pp, Frac-950, and MLVS, adjusting for age, sex, smoking status and baseline measurements of FEV_1 pp, Frac-950 and MLVS, respectively. Stepwise selection method was used for variable selection and Akaike Information Criterion (AIC) for comparing models and choosing the optimal model.

3.4.2 Binary Clinical Measurements

Logistic regression analysis was applied to estimate the associations of plasma biomarkers with the rapid decline of FEV_1 pp, Frac-950, and MLVS with and without adjusting for age, sex, smoking status and baseline measurements of FEV_1 pp, Frac-950 and MLVS, respectively. Stepwise selection method was used for variable selection and Akaike Information Criterion (AIC) for comparing models and choosing the optimal model.

3.4.3 Influential Observations

In this study, the influential observations include the observations those do not fit the current model and those whose removal cause a large change to the model fit.

All statistical procedures were performed with R (version i386 3.1.1).

4.0 RESULTS

4.1 SUBJECT CLINICAL AND DEMOGRAPHIC CHARACTERISTICS

The 2-year follow-up cohort consisted of 313 subjects, including 118 (37.70%) current smokers and 162 (51.76%) males. The mean age was 68 (± 6.09) years, ranging from 42 to 85 years old. FEV_1 pp ranged from 23% to 133% with a mean 85.44% (± 21.47). Frac-950 ranged from 0.001 to 0.38 with a mean of 0.04 (± 0.07). MLVS ranged from 0 to 5 with a mean of 0.95 (± 1.29) (Table 3).

Table 3. Subject Characteristics at Baseline

(N=313)

Characteristic	Mean ± SD or n (%)
Age	68 ± 6.09
Males	162(51.76%)
Current Smoker	118(37.70%)
FEV_1 pp	85.44 ± 21.47
Frac-950	0.04 ± 0.07
MLVS	0.95 ± 1.29

Distribution of continuous changes in FEV_1 pp, Frac-950 and MLVS are shown in Figure 2. Summaries of rapid decline in FEV_1 pp, Frac-950 and MLVS are shown in Table 4.

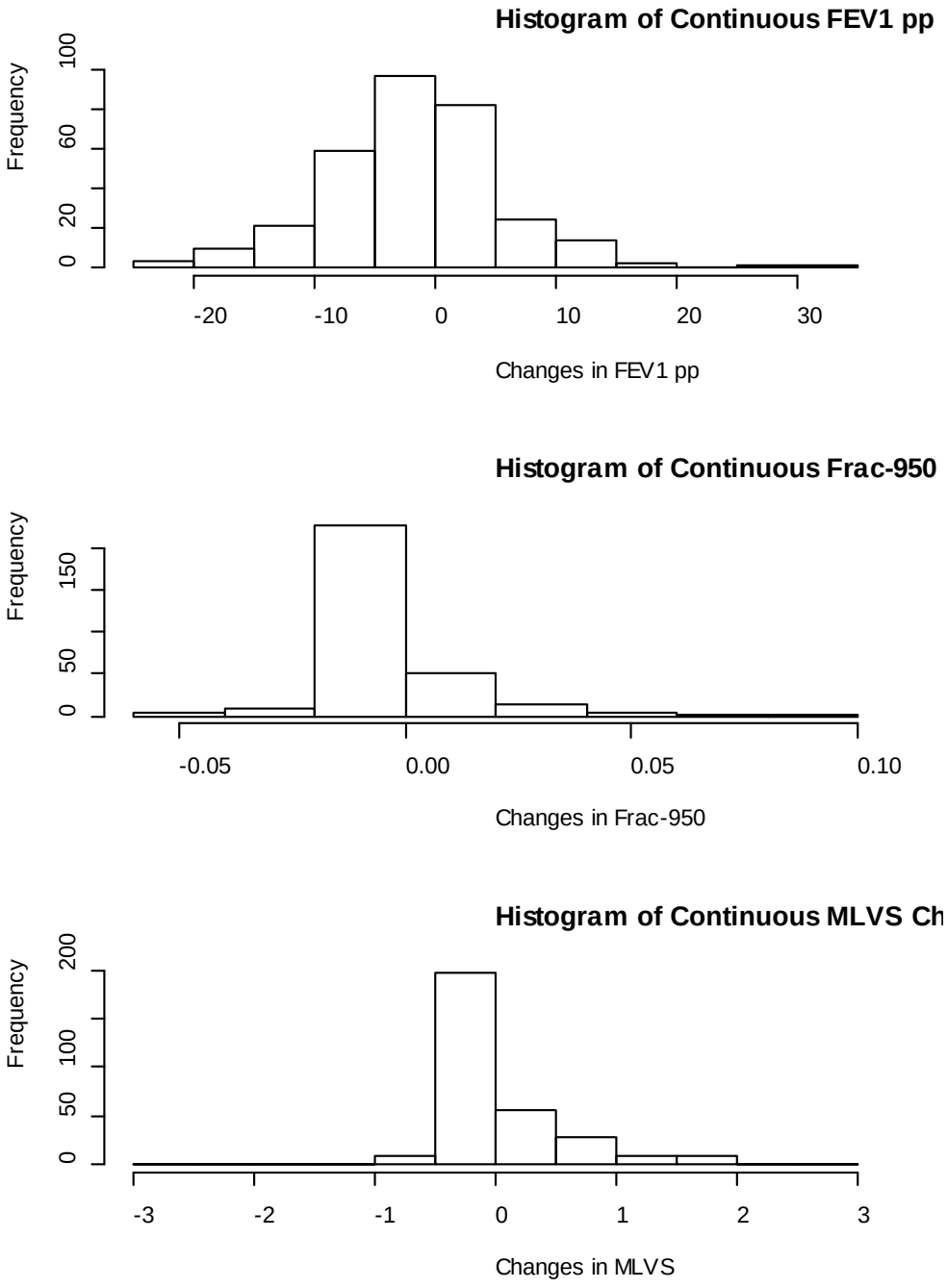

Figure 2. Histogram of Continuous Changes in FEV_1 pp, Frac-950 and MLVS

Table 4. Summary for Rapid Decline of FEV1, Frac-950 and MLVS

	FEV_1 rapid decline	Frac-950 rapid decline	MLVS rapid decline
Yes	92(29.39%)	71(22.68%)	57(18.21%)
No	221(70.61%)	242(77.32%)	256(81.79%)

4.2 PERIPHERAL BLOOD BIOMARKERS

All plasma levels of biomarkers are log-transformed for ease of calculation and comparison. Baseline levels are summarized in Table 5.

Table 5. Summary for Plasma Level of Biomarkers at Baseline

Characteristic (Log-Transform)	Mean ± SD
TRAIL (pg/ml)	4.44 ± 0.38
sFas (pg/ml)	8.96 ± 0.32
SOST (pg/ml)	4.02 ± 0.51
TNFRI (pg/ml)	7.09 ± 0.30
TNFRII (pg/ml)	7.88 ± 0.33
CRP (pg/ml)	14.00 ± 1.25
SAA (pg/ml)	14.73 ± 0.96
sICAM1 (pg/ml)	12.09 ± 0.42
sVCAM1 (pg/ml)	12.45 ± 0.41
MMP1 (pg/ml)	7.65 ± 0.81
MMP7 (pg/ml)	9.27 ± 0.50
TIMP1 (pg/ml)	11.11 ± 0.25
TIMP2 (pg/ml)	11.12 ± 0.20
TIMP4 (pg/ml)	7.14 ± 0.40
CCP (ng/ml)	1.43 ± 0.61
SPD (ng/ml)	4.40 ± 0.55
hPTX3 (pg/ml)	6.19 ± 0.61

4.3 ASSOCIATION BETWEEN CHANGE IN FEV_1 PP AND PLASMA BIOMARKERS

4.3.1 Continuous FEV_1 pp change

Table 6 contains the unadjusted and adjusted results of regressing each biomarker individually on FEV_1 pp change over 2 years. Plasma levels of CRP and MMP1 are statistically significant and positively associated with continuous FEV_1 pp change when adjusting for age, sex, smoking status and FEV_1 pp value when entering the study. With 1 pg/ml increases in the log-transformed plasma level of CRP, the estimated change in FEV_1 pp increases by 0.87 with adjusting for age, sex, smoking status and FEV_1 pp at baseline. Plasma level of TIMP1 shows a positive trend in estimating FEV_1 pp change with adjustment (regression coefficient=2.95, p-value=0.07).

Using the stepwise selection method, the plasma levels of TNFRI, TNFRII, CRP, MMP1 and SPD, together with age, sex, smoking status and FEV_1 pp value when entering in study, best estimated the FEV_1 pp change among the 17 available plasma biomarkers (Table 7). 12.07 % of variance in continuous FEV_1 pp change could be explained by age, sex, smoking status, baseline value of FEV_1 pp and plasma levels of TNFRI, TNFRII, CRP, MMP1 and SPD. 3.40% of variance in continuous FEV_1 pp change could be explained by the selected plasma level of biomarkers (the R-square for estimating FEV_1 pp change by adjustments only is 8.67%). Plasma levels of CRP and MMP1 are positively associated with FEV_1 pp change (regression coefficient=0.85 and 1.08, p-value=0.01 and 0.03, respectively). The higher plasma level of CRP at baseline, the greater the estimated value of FEV_1 pp change. FEV_1 pp at baseline is highly

significantly associated with continuous FEV_1 pp change (regression coefficient=11.73, p-value<0.001).

Table 6. Association between Continuous FEV_1 pp Change and Biomarkers

Biomarker (log-transform)	Unadjusted		Adjusted*	
	Coefficient	P-value	Coefficient	P-value
TRAIL	0.56	0.60	-0.001	0.99
sFas	1.04	0.43	0.17	0.90
SOST	-0.07	0.94	-0.45	0.58
TNFRI	1.50	0.29	1.44	0.29
TNFRII	0.46	0.71	0.33	0.79
CRP	0.55	0.10	0.87	*0.007*
SAA	0.53	0.22	0.58	0.17
sICAM1	-0.02	0.98	0.17	0.86
sVCAM1	-0.35	0.73	-0.13	0.89
MMP1	0.86	0.09	1.15	*0.02*
MMP7	-0.50	0.55	-0.49	0.56
TIMP1	2.52	0.13	2.95	0.07
TIMP2	0.72	0.73	0.71	0.73
TIMP4	-1.29	0.21	-0.80	0.45
CCP	1.02	0.14	0.44	0.55
SPD	0.54	0.48	0.94	0.20
hPTX3	-0.65	0.34	-0.57	0.39

*Adjusted for age, sex, smoking status and FEV_1 pp at baseline.

Table 7. Fitted Model for Estimating Continuous FEV_1 pp Change

Dependent Variable	Independent Variable*	Coef.	P-value	AIC	R-Squared	MSE
FEV1 pp Change	TNFRI	4.09	0.15	1218.76	0.12	46.06
	TNFRII	-0.99	0.11			
	CRP	0.85	*0.01*			
	MMP1	1.08	*0.03*			
	SPD	1.09	0.14			
	Age	-0.11	0.11			
	Male	-0.31	0.69			
	Smoking(Y)	-0.94	0.25			
	FEV1 pp at baseline	11.73	*<0.001*			

*Log-transform applied to plasma level of biomarkers.

There are some observations could influence the selection of plasma biomarkers (solid red points shown in Figure 3). After removing these observations, the plasma level of MMP1 is no longer significant in estimating FEV_1 pp change in the fitted model (regression coefficient=0.57, p-value=0.19), and plasma level of CRP and FEV_1 pp at baseline are still significant positively in estimating FEV_1 pp change (regression coefficient=0.73 and 13.52, p-value=0.01 and <0.001, respectively).

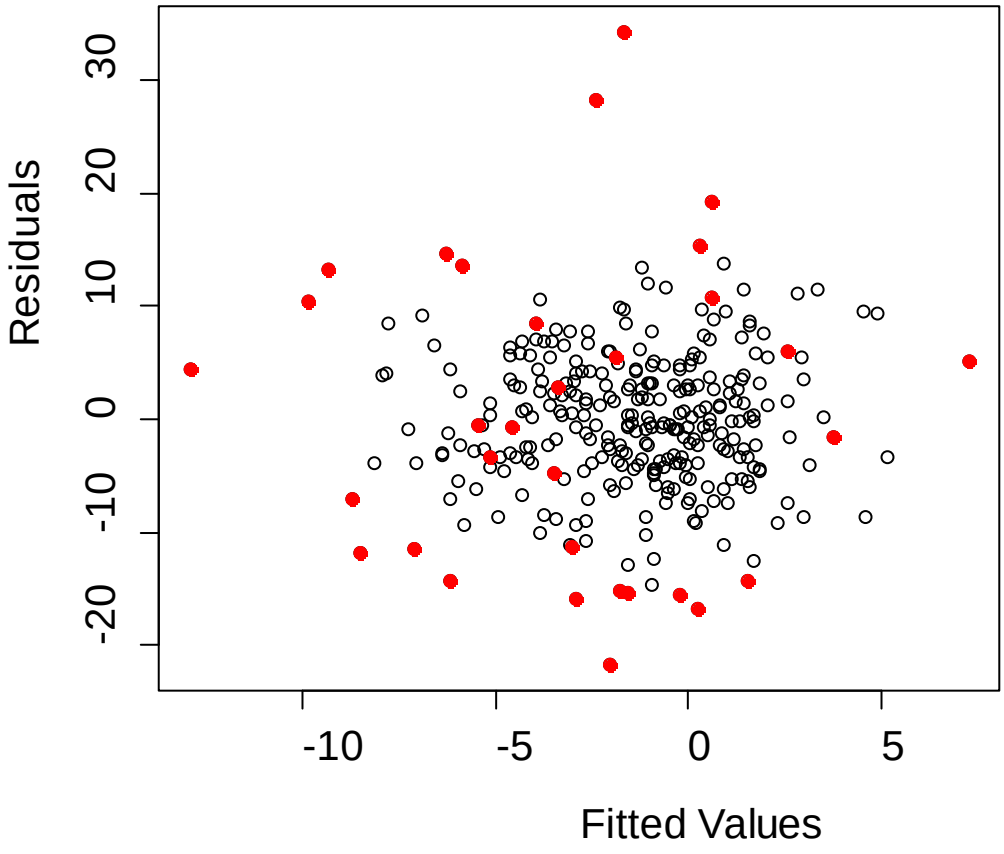

Figure 3. Residual Plot of Fitted Model for Continuous FEV_1 pp Change

4.3.2 FEV_1 rapid decline

Table 8 contains the unadjusted and adjusted results of regressing each biomarker individually on FEV_1 rapid decline over 2 years. When estimating the association between FEV_1 rapid decline and biomarkers one at a time, there are no significant associations observed. Plasma level of CCP shows a trend in association with FEV_1 pp change without adjustment (OR=0.69, p-value=0.06), however, this trend does not show up after adjusting for age, sex, smoking status and FEV_1 pp at baseline (OR=0.82, p-value=0.38).

Table 8. Association between FEV_1 Rapid Decline and Biomarkers

Biomarker (log-transform)	Unadjusted		Adjusted*	
	Odds Ratio	P-value	Odds Ratio	P-value
TRAIL	0.85	0.63	0.95	0.89
sFas	0.75	0.46	0.96	0.92
SOST	0.85	0.52	0.95	0.85
TNFRI	0.68	0.36	0.62	0.30
TNFRII	0.86	0.68	0.85	0.69
CRP	0.94	0.52	0.85	0.12
SAA	0.89	0.37	0.87	0.32
sICAM1	0.92	0.77	0.84	0.57
sVCAM1	1.24	0.47	1.19	0.59
MMP1	0.91	0.56	0.83	0.25
MMP7	1.08	0.76	1.01	0.96
TIMP1	0.68	0.43	0.58	0.30
TIMP2	1.66	0.41	1.89	0.34
TIMP4	1.29	0.40	1.11	0.77
CCP	0.69	0.06	0.82	0.38
SPD	1.00	0.98	0.89	0.63
hPTX3	1.13	0.55	1.12	0.59

*Adjusted for age, sex, smoking status and FEV_1 pp at baseline.

According to stepwise selection method, the plasma levels of CRP, sICAM1 and sVCAM1, together with age, sex, smoking status and FEV_1 pp at baseline, could best estimate

the FEV_1 pp change among the 17 available plasma biomarkers (Table 9). Only plasma level of sVCAM1 shows a positive trend in estimating FEV_1 rapid decline among the selected 3 biomarkers (OR=2.78, p-value=0.07). The association between FEV_1 pp at baseline and FEV_1 rapid decline is significant and for one unit increase in FEV_1 pp at baseline, the odds of rapid decline in FEV_1 (versus no rapid decline) would decrease by 95.76% while holding age, sex, smoking status and plasma levels of the three biomarkers remain the same.

Table 9. Fitted Model for Estimating FEV_1 Rapid Decline

Dependent Variable	Independent Variable*	Odds Ratio	P-value	AIC
FEV_1 Rapid Decline	CRP	0.84	0.14	362.15
	sICAM1	0.44	0.16	
	sVCAM1	2.78	0.07	
	Age	1.02	0.44	
	Male	1.00	0.99	
	Smoking(Y)	1.64	0.08	
	FEV_1 pp at baseline	0.04	*<0.001*	

*Log-transform applied to plasma level of biomarkers.

The ROC curve for the fitted model shows a moderate fit and the area under the curve is 69.53% (Figure 4). Based on the fitted model for FEV_1 rapid decline, the observed and predicted values are shown in Table 10. The fitted model has a high specificity, 93.21%, however, the sensitivity is low, 20.65%.

Table 10. Contingence Table of FEV_1 Rapid Decline

Predicted FEV_1 Rapid Decline	Observed FEV_1 Rapid Decline		Validation Measurement		
	Decline	No Decline	Classification Rate	Sensitivity	Specificity
Decline	19	15	71.88%	20.65%	93.21%
No Decline	73	206			

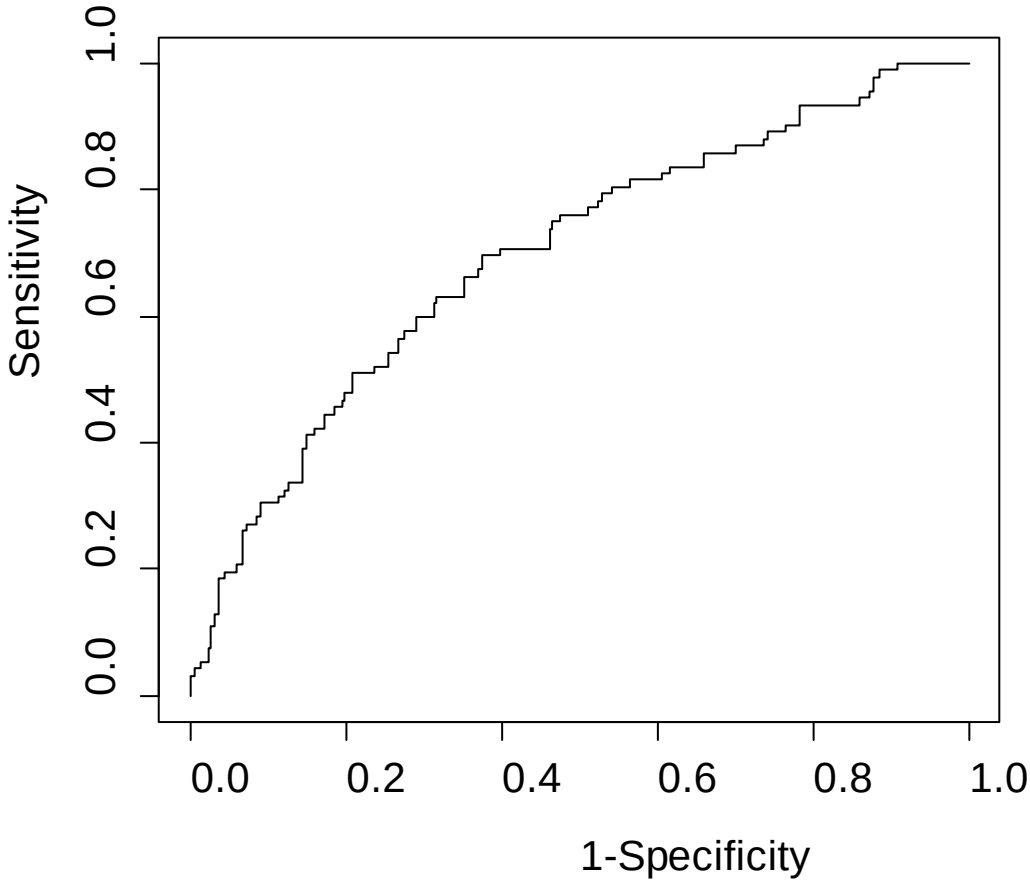

Figure 4. ROC Curve of Fitted Model for FEV_1 Rapid Decline

4.4 ASSOCIATION BETWEEN CHANGE IN QUANTITATIVE MEASUREMENT OF EMPHYSEMA AND PLASMA BIOMARKERS

4.4.1 Continuous Frac-950 change

Table 11 contains the unadjusted and adjusted results of regressing each biomarker individually on Frac-950 change over 2 years. Plasma level of sICAM1 is positively associated with continuous Frac-950 change when adjusting for age, sex, smoking status and Frac-950 at baseline (regression coefficient=0.004, p-value=0.02). When keeping age, sex, smoking status and Frac-950 at baseline constant, 1 pg/ml increase in log-transformed plasma level of sICAM1 increases the continuous Frac-950 change by 0.004. Plasma level of TRAIL, SOST, sVCAM1 and SPD show a positive trend in estimating Frac-950 change with adjustment.

According to stepwise selection method, the plasma levels of TRAIL, sICAM1, TIMP2, CCP and SPD, together with age, sex, smoking status and Frac-950 at baseline, could best estimate the continuous Frac-950 change among the 17 available plasma biomarkers (Table 12). 21.56% of variance in continuous Frac-950 change could be explained by age, sex, smoking status, Frac-950 at baseline and plasma levels of TRAIL, sICAM1, TIMP2, CCP and SPD. 2.54% of variance in continuous Frac-950 change could be explained by the selected plasma level of biomarkers (the R-square for estimating Frac-950 change by adjustments only is 0.19). Plasma level of sICAM1 is positively associated with Frac-950 change (regression coefficient=0.004, p-value=0.02). The higher plasma level of sICAM1 at baseline, the greater the estimated value of Frac-950 change. Plasma level of TRAIL shows a positive trend in estimating Frac-950 change (regression coefficient=0.004, p-value=0.09). Frac-950 at baseline is highly significantly associate with continuous Frac-950 change (regression coefficient=0.11, p-value<0.001).

Table 11. Association between Continuous Frac-950 Change and Biomarkers

Biomarker (log-transform)	Unadjusted		Adjusted*	
	Coefficient	P-value	Coefficient	P-value
TRAIL	0.001	0.59	0.004	0.07
sFas	-0.002	0.46	0.001	0.75
SOST	-0.001	0.92	0.001	0.40
TNFRI	0.004	0.18	0.005	0.06
TNFRII	0.003	0.28	0.004	0.13
CRP	0.001	0.48	0.001	0.39
SAA	0.0003	0.72	0.001	0.41
sICAM1	0.003	0.15	0.004	*0.02*
sVCAM1	0.003	0.18	0.003	0.09
MMP1	0.002	0.09	0.001	0.20
MMP7	-0.001	0.79	0.002	0.36
TIMP1	-0.0003	0.93	0.001	0.88
TIMP2	-0.005	0.21	-0.004	0.34
TIMP4	-0.0004	0.87	-0.0001	0.94
CCP	0.002	0.21	0.002	0.12
SPD	0.003	0.11	0.002	0.10
hPTX3	0.001	0.40	0.002	0.19

*adjusted for age, sex, smoking status and Frac-950 at baseline.

Table 12. Fitted Model for Estimating Continuous Frac-950 Change

Dependent Variable	Independent Variable*	Coef.	P-value	AIC	R-Squared	MSE
Frac-950 Change	TRAIL	0.004	0.09	-2696.17	0.22	0.0002
	sICAM1	0.004	*0.02*			
	TIMP2	-0.006	0.14			
	CCP	0.002	0.15			
	SPD	0.002	0.16			
	Age	0.0001	0.54			
	Male	0.0004	0.78			
	Smoking(Y)	-0.0003	0.86			
	frac-950 at baseline	0.11	*<0.001*			

*Log-transform applied to plasma level of biomarkers.

There are some observations influencing the magnitude of plasma biomarkers (solid red points shown in Figure 5). After removing these observations, the positive trend between plasma level of TRAIL is no longer significant in the fitted model, and plasma level of sICAM1 and Frac-950 at baseline change are significantly positive in estimating Frac-950 change (regression coefficient=0.002 and 0.14, p-value=0.04 and <0.001 respectively).

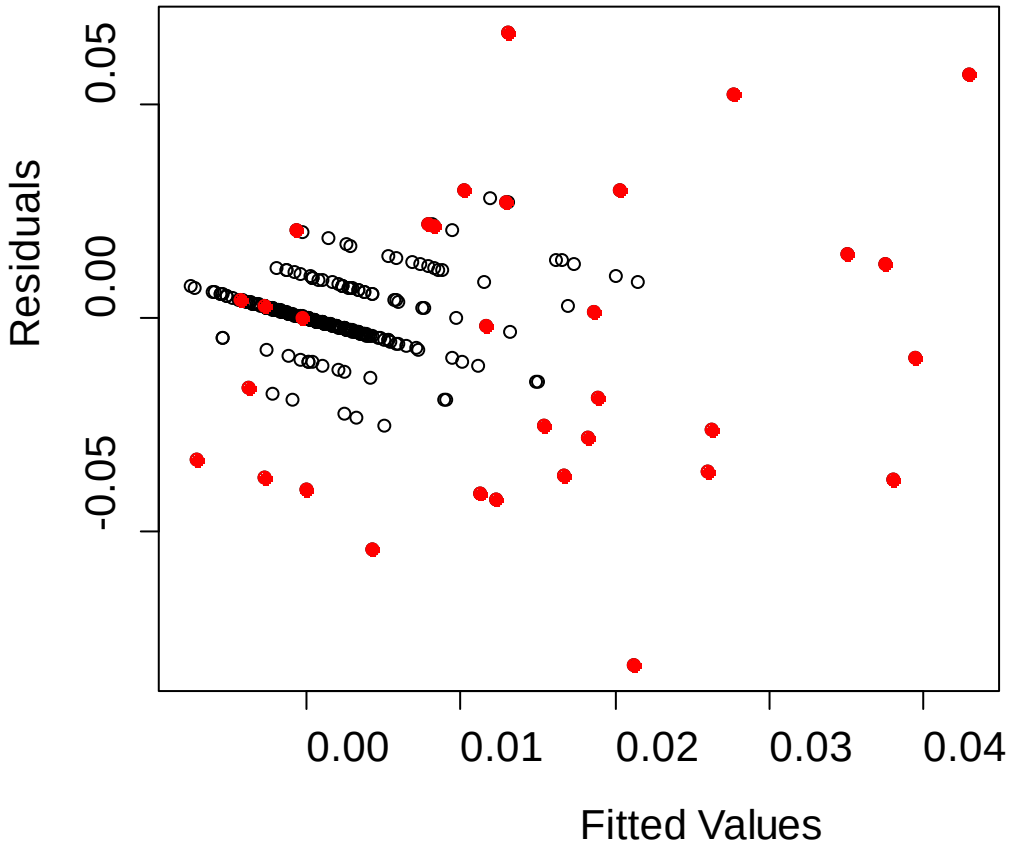

Figure 5. Residual Plot of Fitted Model for Continuous Frac-950 Change

4.4.2 Frac-950 rapid decline

Table 13 contains the unadjusted and adjusted results of regressing each biomarker individually on Frac-950 rapid decline over 2 years. Plasma level of MMP1 is significant positively associated with Frac-950 rapid decline with and without adjusting for age, sex, smoking status and Frac-950 at baseline (OR=1.60 and 1.67, p-value=0.01 and 0.003, respectively. Table 13). For 1 pg/ml increase in log-transformed plasma level of MMP1 at baseline, the odds of Frac-950 rapid decline increase by 59.52% and 66.94% with and without adjustment respectively. There is a negative trend between plasma level of TRAIL and Frac-950 rapid decline without adjustment (OR=0.53, p-value=0.07) but this trend is not observed after adjustment (OR=0.92, p-value=0.83).

According to stepwise selection method, the plasma levels of sICAM1, sVCAM1, MMP1 and TIMP2, together with age, sex, smoking status and Frac-950 at baseline, could best estimate Frac-950 rapid decline among the 17 available plasma biomarkers (Table 14). Plasma levels of sICAM1 and MMP1 are significantly positively associated with Frac-950 rapid decline among the four selected plasma biomarkers (OR=4.46 and 1.59, p-value=0.02 and 0.02, respectively). The odds ratio for Frac-950 at baseline is very large and needs to be further investigated

Table 13. Association between Frac-950 Rapid Decline and Biomarkers

Biomarker (log-transform)	Unadjusted		Adjusted*	
	Odds Ratio	P-value	Odds Ratio	P-value
TRAIL	0.53	0.07	0.92	0.83
sFas	0.56	0.18	0.65	0.39
SOST	0.67	0.12	0.89	0.71
TNFRI	1.60	0.30	1.55	0.39
TNFRII	1.44	0.37	1.39	0.46
CRP	1.17	0.15	1.25	0.09
SAA	1.03	0.83	1.15	0.38
sICAM1	1.10	0.77	1.61	0.19
sVCAM1	0.94	0.86	1.00	0.99
MMP1	1.67	*0.003*	1.60	*0.01*
MMP7	1.09	0.76	1.54	0.19
TIMP1	1.61	0.38	1.54	0.47
TIMP2	0.59	0.45	0.36	0.20
TIMP4	1.03	0.93	0.93	0.85
CCP	1.28	0.29	1.13	0.67
SPD	1.41	0.17	1.47	0.19
hPTX3	1.24	0.33	1.33	0.24

*Adjusted for age, sex, smoking status and Frac-950 at baseline.

Table 14. Fitted Model for Estimating Frac-950 Rapid Decline

Dependent Variable	Independent Variable*	Odds Ratio	P-value	AIC
Frac-950 Rapid Decline	sICAM1	4.46	*0.02*	238.28
	sVCAM1	0.29	0.07	
	MMP1	1.59	*0.02*	
	TIMP2	0.28	0.12	
	Age	1.07	*0.009*	
	Male	1.73	0.09	
	Smoking(Y)	0.68	0.26	
	Frac-950 at baseline	6453931.26	*<0.001*	

*Log-transform applied to plasma level of biomarkers.

The ROC curve for the fitted model shows a moderate fit and the area under the curve is 83.81% (Figure 6). Based on the fitted model for Frac-950 rapid decline, the observed and predicted values are shown in Table 15. The fitted model for Frac-950 rapid decline has a high specificity but a low sensitivity (specificity=95.45% and sensitivity=23.94%).

Table 15. Contingence Table of Frac-950 Rapid Decline

Frac-950 Rapid Decline	Frac-950 Rapid Decline		Validation Measurement		
	Decline	No Decline	Classification Rate	Sensitivity	Specificity
Decline	17	11	79.23%	23.94%	95.45%
No Decline	54	231			

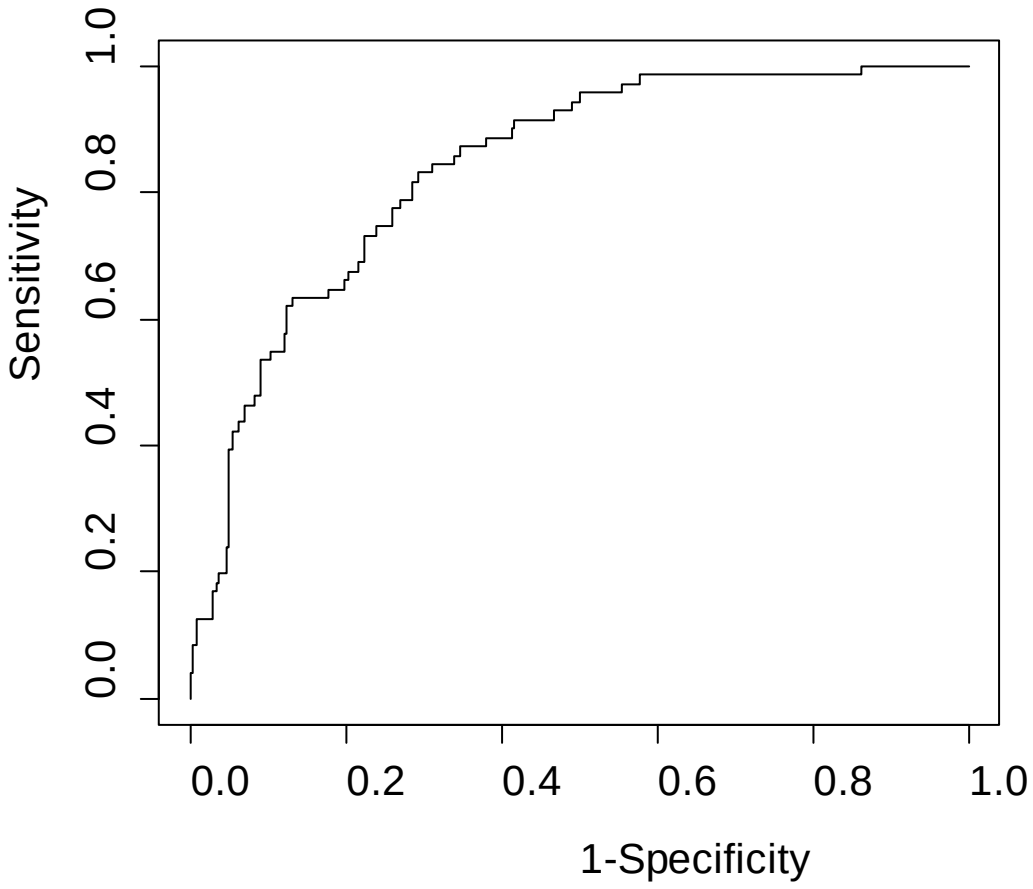

Figure 6. ROC Curve of Fitted Model for Frac-950 Rapid Decline

4.4.3 Continuous MLVS change

Table 16 contains the unadjusted and adjusted results of regressing each biomarker individually on MLVS change over 2 years. Plasma levels of TRAIL, SOST and sICAM1 are significant negatively associated with continuous MLVS change (all regression coefficient<0 and all p-values<0.05), while plasma level of TIMP2 is positively associated with continuous MLVS change (regression coefficient=0.30 and 0.32, p-value=0.02 and 0.03, respectively), with and without adjusting for age, sex, smoking status and MLVS at baseline. Plasma level of sVCAM1 shows a negative trend in estimating MLVS change with and without adjustment (regression coefficient=-0.11 and -0.14, p-value=0.06 and 0.05, respectively), and plasma level of TIMP4 show a positive trend in estimating MLVS change without adjustment (regression coefficient=0.13, p-value=0.08).

According to stepwise selection method, the plasma levels of TRAIL, sFas, SOST, sICAM1, TIMP1, TIMP2 and TIMP4, together with age, sex, smoking status and MLVS at baseline, could best estimate the continuous MLVS change among the 17 available plasma biomarkers (Table 17). 35.3% of variance in continuous Frac-950 change could be explained by age, sex, smoking status, MLVS at baseline and plasma levels of TRAIL, sFas, SOST, sICAM1, TIMP1, TIMP2 and TIMP4. 4.8% of variance in continuous MLVS change could be explained by the selected plasma level of biomarkers. Only plasma levels of TRAIL and TIMP2 of the selected plasma level of biomarkers are significantly associated with MLVS change with opposite direction (regression coefficient=-0.15 and 0.34, p-value=0.03 and 0.02, respectively). The lower plasma level of TRAIL at baseline, the greater the estimated value of MLVS change. While the higher plasma level of TIMP2 at baseline, the greater the estimated value of MLVS change. Plasma levels of sICAM1 and TIMP1show negative trends in estimating MLVS change

while plasma level of Fas shows a positive trend (regression coefficient=-0.13, -0.23 and 0.1527, p-value=0.07, 0.05 and 0.06, respectively). MLVS at baseline is significant positively associate with continuous MLVS change (regression coefficient=0.22, p-value<0.001).

Table 16. Association between Continuous MLVS Change and Biomarkers

Biomarker (log-transform)	Unadjusted		Adjusted*	
	Coefficient	P-value	Coefficient	P-value
TRAIL	-0.27	*<0.001*	-0.15	*0.03*
sFas	0.03	0.75	0.11	0.16
SOST	-0.25	*<0.001*	-0.15	*0.003*
TNFRI	0.06	0.56	0.06	0.51
TNFRII	0.06	0.53	0.04	0.57
CRP	-0.01	0.74	-0.02	0.23
SAA	-0.03	0.39	-0.03	0.28
sICAM1	-0.20	*0.005*	-0.18	*0.003*
sVCAM1	-0.14	0.05	-0.11	0.06
MMP1	0.03	0.48	-0.02	0.58
MMP7	-0.05	0.45	-0.04	0.45
TIMP1	-0.05	0.68	-0.08	0.46
TIMP2	0.32	*0.03*	0.30	*0.02*
TIMP4	0.13	0.08	0.07	0.29
CCP	0.05	0.31	0.04	0.32
SPD	-0.01	0.89	-0.05	0.31
hPTX3	-0.01	0.78	-0.03	0.49

*Adjusted for age, sex, smoking status and MLVS at baseline.

There are some observations could influent the significance of plasma biomarkers (solid red points shown in Figure 7). After removing these observations, the plasma level of TRAIL is no longer significant in estimating MLVS change in the fitted model (regression coefficient=-0.06, p-value=0.23), the plasma levels of SOST and TIMP1 become significant in estimating MLVS change in the fitted model (regression coefficient=-0.09 and -0.18, p-value=0.04 and 0.0465, respectively), and plasma level of TIMP2 and MLVS at baseline are still significant

positively in estimating MLVS change in the fitted model (regression coefficient=0.33 and 0.17, p-value=0.004 and <0.001, respectively).

Table 17. Fitted Model for Estimating Continuous MLVS Change

Dependent Variable	Independent Variable*	Coef.	P-value	AIC	R-Squared	MSE
MLVS Change	TRAIL	-0.15	*0.03*	-521.74	0.35	0.18
	sFas	0.15	0.06			
	SOST	-0.09	0.14			
	sICAM1	-0.13	0.07			
	TIMP1	-0.23	0.05			
	TIMP2	0.34	*0.02*			
	TIMP4	0.11	0.11			
	Age	0.0004	0.92			
	Male	-0.01	0.78			
	Smoking(Y)	0.06	0.26			
	MLVS at baseline	0.22	*<0.001*			

*Log-transform applied to plasma level of biomarkers.

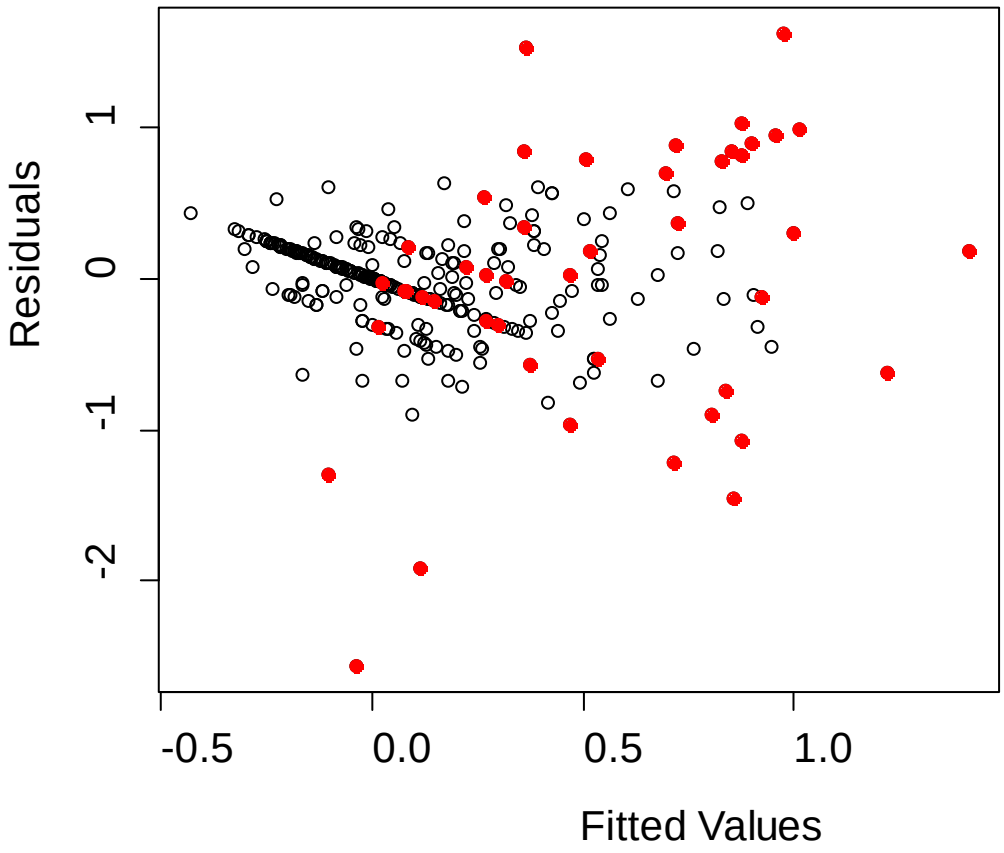

Figure 7. Residual Plot of Fitted Model for MLVS Change

4.4.4 MLVS rapid decline

Table 18 contains the unadjusted and adjusted results of regressing each biomarker individually on MLVS rapid decline over 2 years. Plasma levels of TRAIL, SOST and sICAM1 is significant negatively associated with MLVS rapid decline (all OR<1, all p-value<0.05) while plasma level of TIMP2 is significant positively associated with MLVS rapid decline with and without adjusting for age, sex, smoking status and MLVS at baseline (OR=8.81 and 4.12, p-

value=0.03 and 0.04, respectively. Table 18). There is a negative trend between plasma level of sVCAM1 and MLVS rapid decline with and without adjustment (OR=0.41 and 0.51, p-value=0.05 and 0.06, respectively).

Table 18. Association between MLVS Rapid Decline and Biomarkers

Biomarker (log-transform)	Unadjusted		Adjusted	
	Odds Ratio	P-value	Odds Ratio	P-value
TRAIL	0.27	*0.001*	0.34	*0.04*
sFas	0.96	0.92	2.35	0.18
SOST	0.27	*<0.001*	0.30	*0.001*
TNFRI	0.84	0.72	0.64	0.50
TNFRII	1.06	0.89	0.97	0.95
CRP	0.96	0.71	0.85	0.32
SAA	0.83	0.23	0.67	0.06
sICAM1	0.40	*0.009*	0.28	*0.006*
sVCAM1	0.51	0.06	0.41	0.05
MMP1	1.04	0.81	0.83	0.40
MMP7	0.70	0.23	0.53	0.13
TIMP1	0.63	0.42	0.44	0.28
TIMP2	4.12	*0.04*	8.81	*0.03*
TIMP4	1.50	0.26	0.91	0.85
CCP	1.17	0.53	1.09	0.81
SPD	1.17	0.56	0.91	0.83
hPTX3	1.07	0.77	1.04	0.91

*Adjusted for age, sex, smoking status and MLVS at baseline.

According to stepwise selection method, plasma levels of TRAIL, sFas, SOST, TIMP1, and TIMP2, together with age, sex, smoking status and MLVS at baseline, could best estimate MLVS rapid decline among the 17 available plasma biomarkers (Table 19). Plasma levels of SOST and TIMP1 are significant negatively (OR=0.33 and 0.12, p-value=0.005 and 0.03, respectively), and plasma level of TIMP2 and MLVS at baseline are significant positively in

estimating MLVS rapid decline in the fitted model (OR=25.74 and 4.32, p-value=0.01 and <0.001, respectively).

Table 19. Fitted Model in Estimating MLVS Rapid Decline

Dependent Variable	Independent Variable*	Odds Ratio	P-value	AIC
MLVS Rapid Decline	TRAIL	0.33	0.07	173.83
	sFas	4.00	0.05	
	SOST	0.33	*0.005*	
	TIMP1	0.12	*0.03*	
	TIMP2	25.74	*0.009*	
	Age	1.04	0.33	
	Male	0.62	0.28	
	Smoking(Y)	1.84	0.19	
	MLVS at baseline	4.32	*<0.001*	

*Log-transform applied to plasma level of biomarkers.

The ROC curve for the fitted model shows a good fit and the area under the curve is 93.49% (Figure 8). Based on the fitted model for MLVS rapid decline, the observed and predicted values are shown in Table 20. The fitted model has a high specificity and a moderate sensitivity in estimating MLVS rapid decline (specificity=96.88% and sensitivity=61.40%).

Table 20. Contingence Table of MLVS Rapid Decline

MLVS Rapid Decline	MLVS Rapid Decline		Validation Measurement		
	Decline	No Decline	Classification Rate	Sensitivity	Specificity
Decline	35	8	90.42%	61.40%	96.88%
No Decline	22	248			

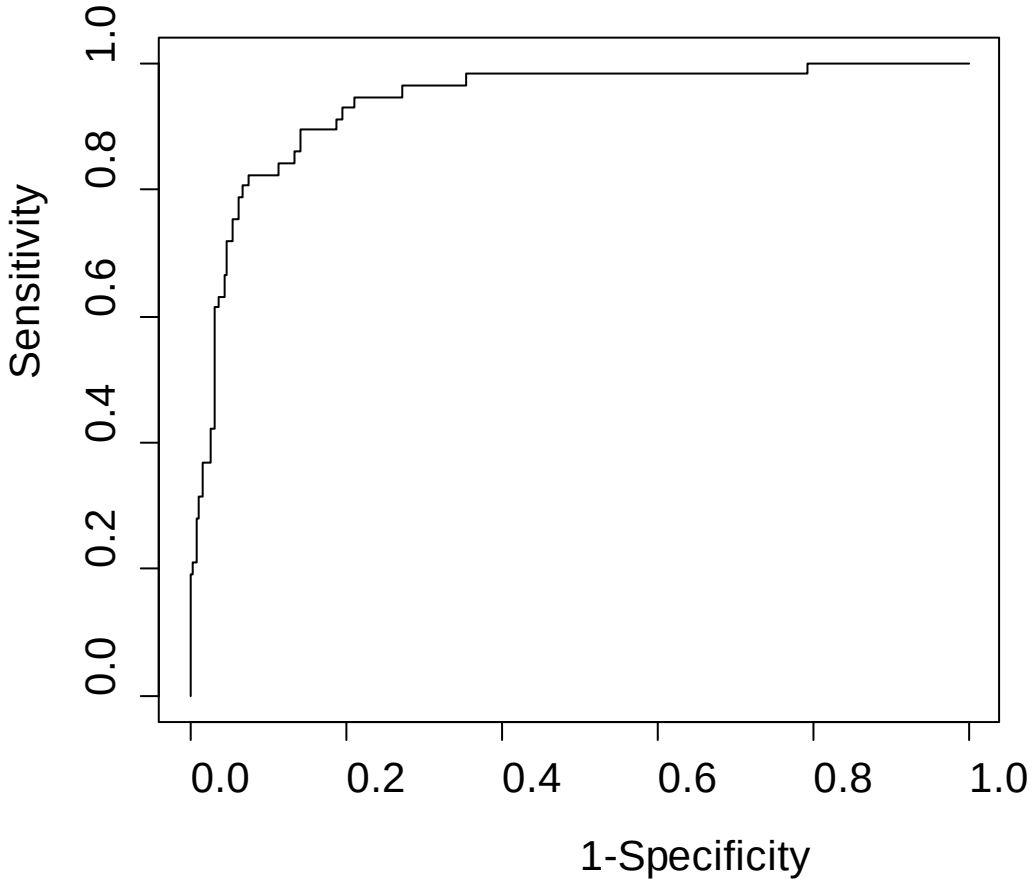

Figure 8. ROC Curve of Fitted Model for MLVS Rapid Decline

5.0 DISCUSSION

COPD is a progressive disease affecting a large population. During the past decade, the mortality of COPD has continued to increase. Due to the key feature of COPD that accelerated decline in FEV_1, early treatment to the rapid lung function decliners will reduce disease burden and disease exacerbation for patients.

In this study, we identified and selected the plasma biomarkers in estimating both continuous change and rapid decline in FEV_1, Frac-950 and MLVS. Table 21, 22 and 23 are summaries for selected plasma biomarkers in estimating pulmonary measurements.

We find there are common plasma biomarkers in estimating different phenotype of COPD progression and the associations are consistent between different phenotypes. A higher plasma level of sVCAM1 indicates a greater FEV_1 rapid decline while a lower level indicates a greater Frac-950 rapid decline. A lower plasma level of sICAM1 indicates a greater FEV_1 rapid decline while a higher level indicates a greater Frac-950 rapid decline. These results are seem to be contradicted. They are consistent in actual since higher FEV_1 and lower Frac-950 indicate a healthier lung.

We also find there are common plasma biomarkers with inconsistent association between phenotypes. Higher plasma level of SPD indicates both greater continuous change in FEV_1 pp and Frac-950. Higher plasma level of MMP1 indicates greater FEV_1 pp change and greater Frac-950 rapid decline.

Frac-950 and MLVS are two measurements for emphysema. For the same plasma biomarker, it should have a consistent relationship with these two measurements. However, it is not always the case. We observe higher plasma level of sICAM1 associates with greater continuous change and a greater rapid decline in Frac-950 while lower value associates with greater MLVS change. Similar association is observed for plasma level of TRAIL.

We observed there were biomarkers consistently in estimating COPD progression within the same phenotype. Plasma level of sICAM1 is positively associated with both continuous change and rapid decline in Frac-950, and plasma levels of TRAIL, SOST and TIMP1 are negatively while plasma levels of sFas and TIMP2 are positively associated with both continuous change and rapid decline in MLVS. We also observed there was inconsistent relationship within the same phenotype. Higher levels of CRP indicate greater FEV_1 pp change while lower levels indicate greater FEV_1 rapid decline. This contradiction may due to the cut-off point chosen for FEV_1 rapid decline. Besides, we noticed the selected plasma biomarkers were almost the same in estimating MLVS change and rapid decline.

Table 21. Significant association between FEV_1 and biomarkers

Biomarker (log-transform)	Continuous FEV_1 pp Change			FEV_1 Rapid Decline		
	Unadjusted	Adjusted*	Fitted Model	Unadjusted	Adjusted*	Fitted Model
TNFRI	(+)	(+)	(+)			
TNFRII	(+)	(+)	(-)			
CRP	(+)	*(+)*	*(+)*	(-)	(-)	(-)
MMP1	(+)	*(+)*	*(+)*			
SPD	(+)	(+)	(+)			
sICAM1				(-)	(-)	(-)
sVCAM1				(+)	(+)	(+)

*Adjusted for age, sex, smoking status and FEV_1 pp at baseline. (+)/(-) signs show positive/negative association and significant ones are marked in red and italics.

Table 22. Significant association between Frac-950 and biomarkers

Biomarker (log-transform)	Continuous Frac-950 Change			Frac-950 Rapid Decline		
	Unadjusted	Adjusted*	Fitted Model	Unadjusted	Adjusted*	Fitted Model
TRAIL	(+)	(+)	(+)			
sICAM1	(+)	*(+)*	*(+)*	(+)	(+)	*(+)*
TIMP2	(-)	(-)	(-)	(-)	(-)	(-)
CCP	(+)	(+)	(+)			
SPD	(+)	(+)	(+)			
sVCAM1				(-)	(+)	(-)
MMP1				(+)	(+)	*(+)*

*Adjusted for age, sex, smoking status and Frac-950 at baseline. (+)/(-) signs show positive/negative association and significant ones are marked in red and italics.

Table 23. Significant association between MLVS and biomarkers

Biomarker (log-transform)	Continuous MLVS Change			MLVS Rapid Decline		
	Unadjusted	Adjusted*	Fitted Model	Unadjusted	Adjusted*	Fitted Model
TRAIL	*(-)*	*(-)*	*(-)*	*(-)*	*(-)*	(-)
sFas	(+)	(+)	(+)	(-)	(+)	(+)
SOST	*(-)*	*(-)*	(-)	*(-)*	*(-)*	*(-)*
sICAM1	*(-)*	*(-)*	(-)			
TIMP1	(-)	(-)	(-)	(-)	(-)	*(-)*
TIMP2	*(+)*	*(+)*	*(+)*	*(+)*	*(+)*	*(+)*
TIMP4	(+)	(+)	(+)			

*Adjusted for age, sex, smoking status and MLVS at baseline. (+)/(-) signs show positive/negative association and significant ones are marked in red and italics.

We find that plasma levels of TIMP1 and TIMP2 have opposite association with continuous change and rapid decline in MLVS. Although TIMP1 and TIMP2 come from the same family, tissue inhibitor of metalloproteinase, they render based on different protein and have different function. Besides inhibiting matrix metalloproteinases, TIMP2 could suppress the proliferation of endothelial cells. Endothelium involve in inflammation [97]. Therefore, plasma level of TIMP1 and TIMP2 may have different association with the same COPD phenotype, MLVS.

We find the baseline values of FEV_1 pp, Frac-950 and MLVS play an active and important role in estimating their corresponding change or rapid decline. The R-squares by baseline values are much greater than the R-squares by selected plasma biomarkers.

We noticed that the plasma biomarkers selected to estimate FEV_1 were almost different from those selected to estimate Frac-950 and MLVS. The biomarkers for estimating FEV_1 may more related to small airway disease while the biomarkers for Frac-950 and MLVS are related to parenchymal destruction.

Our results are consistent with existing published results of associations between plasma biomarkers and inflammation. Plasma level of TRAIL is significantly higher in COPD patients compared to healthy group (50.17±17.70 versus 42.09±15.49 pg/ml, p-value=0.03) [98]. Enhanced level of MMP1 is observed in gingival inflammation [99]. Increased sFas levels are associated with pulmonary fibrosis and bronchiolitis [100, 101]. Serum level of ICAM1 is elevated in vascular inflammation [102] Decreased TIMP1 levels are associated with COPD exacerbation (from 3.5 microg/g to 1.5 microg/g, $p<0.05$) [103]. Low concentrations of CCP are associated with accelerated FEV_1 decline (4.4 mL/year additional FEV_1 decline, p=0·0014) [104].

However, we also have contradictory results with existing literature. Elevated level of CRP is associated with increased COPD hospitalization and death (hazard ratio=1.4 and 2.2) [105]. Serum level of VCAM1 is elevated in vascular inflammation [104]. Higher levels of TNFRI is associated with ventilator-associated pneumonia (regression coefficient=8.9, p-value=0.01) [106]. High circulating SOST indicates a greater risk of osteoporosis-related fractures (p-value<0.001) which has a higher prevalence in COPD patients [35, 107]. High

concentrations of CRP are associated with accelerated FEV_1 decline (30 mL/year additional FEV_1 decline, $p<0{\cdot}001$) [108]. Further study is needed to discuss the inconsistence.

This study has two strengths. The plasma biomarkers are selected based on pathology of COPD, including lung specific proteins, inflammatory protein and proteinase, and apoptosis related cytokines. These selected biomarkers are proven to be directly or indirectly associated with inflammation. In addition, we considered biomarker related to COPD comorbidity, osteoporosis.

There are limitations in this study. COPD is a disease with systematic inflammation, involving many plasma biomarkers. In this study, only 17 biomarkers' relationships with COPD progression are discussed. The selected biomarkers to predict COPD progression from the available 17 biomarkers could only explain a small portion of variation. Identifying more plasma biomarkers associated with COPD will make the progression prediction more accurate. In addition, the measurements may have noise. Our 2-year follow up study only measured twice which may be inaccurate or insufficient in measuring COPD progression, making association and variable selection vary.

Identify the COPD patients with rapid decline in different COPD phenotypes based on more robust models by correctly defining the rapid decline in COPD phenotypes and including more biomarkers and repeated measurements in the future study could improve the accuracy on finding target population.

6.0 PUBLIC HEALTH IMPACT

COPD is a systemic disease with manifestations affecting lungs and extra-pulmonary organs. COPD and its comorbidities increase hospitalizations, morbidity and mortality, and adversely impacts quality of life. Identifying patients who will have a rapid decline in lung function prior to the decline is necessary to provide aggressive treatment to the appropriate patients. Plasma biomarkers, which are inexpensive and easily obtained, could be used to identify those with a high likelihood of rapid decline.

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